

Gene Expression and Promoter Methylation Status of VHL, Runx-3, E-cadherin, P15 and P16 Genes During EPO-Mediated Erythroid Differentiation of CD34+ Hematopoietic Stem Cells

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Abstract

Background: VHL (von Hippel-Lindau), Runx-3 (Runt-related transcription factor 3), E-cadherin (Epithelial cadherin), P15 (INK4a, cyclin dependent kinase inhibitor), and P16 (INK4b) genes are essential in hematopoiesis. The aim of this study was to explore the correlation between gene expression and promoter methylation in CD34+ stem cells before and after differentiation to erythroid lineage.

Materials and Methods: CD34+ hematopoietic stem cells were separated from umbilical cord blood using MidiMacs (positive selection) system. Expanded CD34+ stem cells were differentiated into erythroid lineage with human recombinant erythropoietin (EPO). DNA extraction was done by QIAamp DNA Mini Kit. RNA was extracted using RNase Mini plus Kit. MSP (Methylation specific PCR) technique was done for methylation assay. Methylation status and expression assay was done for VHL, Runx-3, E-cadherin, P15, and P16 genes on both CD34+ stem cells and differentiated erythroid cells.

Results: The results showed that, before differentiation, P15 had comparative methylation pattern and average expression and it remained unchanged after differentiation ($p=0.01$). concerning P16, results revealed no methylation pattern and complete expression in absence of EPO and with EPO it changed to comparative status ($p=0.01$). E-cad and Runx-3 genes had relative methylation pattern and fully expression before and after differentiation but their expression after that, was increased and decreased Respectively ($p=0.04$). VHL gene had no significant methylation status before or after differentiation and its expression was complete ($p=0.01$).

Conclusion: The obtained results indicated that promoter methylation of P15, P16, VHL, Runx3 and E-cad was one of the definitive expression control mechanism of these genes.

Key words: Differentiation, EPO, Hematopoietic Stem Cells

Introduction

Studies about cell differentiation have led to access general mechanisms of regulating and specific factors of gene expression. In fact intracellular signaling and gene expression regulators are the most impressive factor for primary

differentiation process in the cell (1). So the requirement for specific elements like cell cycle controlling molecules or cytokines is quite understandable. In mammalian cells DNA is methylated only at cytosine located 5' to guanosine in CpG